CHLOROPLAST DNA EVOLUTION AND ADAPTIVE RADIATION IN THE HAWAIIAN SILVERSWORD ALLIANCE (ASTERACEAE–MADIINAE)[1]

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ABSTRACT

Chloroplast DNA (cpDNA) restriction site variation among 24 of 27 extant species of the Hawaiian silversword alliance (*Argyroxiphium, Dubautia, Wilkesia*) was studied to enhance understanding of phylogenetic patterns and evolutionary processes in this outstanding example of insular adaptive radiation. Analysis with 16 restriction endonucleases revealed a total of 55 restriction site differences, 36 being shared by a subset of at least two taxa. In pairwise comparisons among these taxa, cpDNA divergence ranged from 0 to 0.46% of nucleotides. Chloroplast DNA divergence within *Dubautia* exceeded that of all intergeneric comparisons. A strict consensus tree from Wagner parsimony analyses of this variation was topologically congruent with evolutionary resolutions from Dollo parsimony and the Fitch-Margoliash genetic distance algorithm. Conflict between these outcomes and results from a phenetic analysis suggest a cpDNA molecular clock is not operating among these taxa. The cpDNA consensus phylogeny indicates monophylesis of the silversword alliance, in accord with biosystematic and cytogenetic evidence. These data further suggest: (1) rapid radiation of lineages early in the history of the silversword alliance; (2) few successful interisland migrations that led to new species lineages; (3) a unidirectional, older-to-younger island pattern of migration within the $n = 13$ *Dubautia* group; (4) striking inconsistency between relative rates of morphological and cpDNA evolution among *Wilkesia* and five Kauaian *Dubautia* species; and (5) hybrid speciation or extensive introgression among certain *Argyroxiphium* and *Dubautia* taxa. These phylogenetic conclusions require testing with molecular analyses of nuclear DNA to clarify the extent to which hybridization has influenced the evolution of this reproductively and genetically cohesive group of plants.

Prominent examples of insular adaptive radiation are widely acknowledged as exceptional demonstrations of organismic evolution. Indeed, these groups of seemingly closely related species are characterized by levels of phenotypic diversity otherwise associated with higher taxa (e.g., Hawaiian Drepanidae, Galápagos Geospizinae). Evolutionary studies of such systems hold great potential to provide unique insights into mechanisms of evolution, origins of major organismic lineages, and genetic bases of conspicuous or fundamental characteristics.

Despite extensive documentation of island plant life (e.g., Carlquist, 1965, 1974), few biosystematic or "experimental" data from insular floras are available. For example, only a small fraction of the Hawaiian archipelago flora has been thus examined to any degree (Carr, 1987). This is unfortunate, given the increasingly precarious position of island ecosystems and the importance of understanding phylogeny for evolutionary interpretations.

In plants, molecular phylogenetic approaches employing DNA appear to be especially suited to the study of insular adaptive radiation. Previous investigations of continental plant groups using chloroplast DNA (cpDNA) have revealed convincing evidence of relationships obscured by morphological evolution (reviewed in Sytsma & Smith, 1988; Palmer et al., 1988). Molecular insight is needed in studies of island species given their apparent potential for rapid morphological change, parallelism, and hybridization.

The Hawaiian silversword alliance represents an

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[1] Supported in part by grants from the National Science Foundation (BSR-8715270: to B. Baldwin and D. Kyhos) and the National Academy of Sciences, through Sigma Xi (to B. Baldwin). We thank S. Bainbridge, J. Canfield, B. Gagné, R. Hobdy, A. Medeiros, J. Obata, S. Perlman, E. Powell, R. Robichaux, L. Stemmermann, and L. Walker for field assistance; R. Jansen, J. Palmer, and K. Sytsma for cpDNA clones; J. Strother and K. Sytsma for helpful reviews; S. Martens for computer assistance; and G. Carr for field assistance, review of the manuscript, and generous cooperation.

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ANN. MISSOURI BOT. GARD. 77: 96–109. 1990.

ideal island plant assemblage for DNA systematic study. Comprising only 28 species in three distinctive genera (*Argyroxiphium*, *Dubautia*, and *Wilkesia*), this manageably small group spans an exceptional range of diversity in morphological, anatomical, and ecological characteristics (Carr, 1985). In fact, Carlquist (1974) regarded the silversword alliance as the most outstanding example of angiosperm adaptive radiation in the Hawaiian archipelago.

The extensive background of evolutionary research on these plants includes anatomical (Carlquist, 1957, 1958a, b, 1959a, b; Kim, 1987), cytogenetic (Carr & Kyhos, 1981, 1986), isozymic (Witter, 1986; Witter & Carr, 1988), ecophysiological (Robichaux, 1984, 1985; Robichaux & Canfield, 1985), chemosystematic (Crins et al., 1988), and breeding system (Carr et al., 1986) investigations. These studies clearly delimit Hawaiian Madiinae and indicate their evolutionary cohesiveness. For example, artificial and natural hybridization have linked nearly all species, the F_1 generations being of at least marginal fertility in every instance (Carr & Kyhos, 1981, 1986). Most importantly for DNA phylogenetic analysis, a closely related lineage that is ancestral to the silversword alliance has been identified by chloroplast DNA investigations and interspecific hybridization involving perennial North American Madiinae (Baldwin, 1989). These studies led to recognition of two outgroup species with close affinities to Hawaiian Madiinae: *Madia bolanderi* and *Raillardiopsis muirii*.

In this paper, we present results from a DNA systematic study of restriction site polymorphisms in the silversword alliance. Chloroplast DNA (cpDNA) was selected for analysis for three reasons: (1) evolutionary interpretation of uniparentally inherited cpDNA is essentially free from the complexities resulting from sexual processes encountered in nuclear DNA (reviewed in Birky, 1988); (2) unlike plant mitochondrial DNA, cpDNA is in most cases structurally conservative and evolves more rapidly by point mutations (Palmer, 1985), facilitating restriction site analysis; and (3) all previous genetic studies of the silversword alliance have focused on the nuclear genome or its expression. A uniparental phylogeny was desired to resolve better the role of hybridization in the evolution of these interfertile species.

MATERIALS AND METHODS

Fresh leaves were field- or greenhouse-collected from representatives of one to five populations of 24 of the 27 extant silversword alliance species and from two outgroup taxa of North American Madiinae, *Madia bolanderi* and *Raillardiopsis muirii* (Table 1). Leaves were ground in liquid N_2 with mortar and pestle. Total DNA was isolated from these leaf N_2 powders and purified twice on cesium chloride gradients using a modified procedure of Palmer (1986), omitting separation of organelles. Mercaptoethanol was increased up to 1% in the extraction buffer to increase the solubility of mucilaginous carbohydrates. DNA aliquots from one or two populations of each species were digested with each of 16 restriction endonucleases recognizing four, five, or six nucleotide-pair sites (Table 3). "Six-cutter" enzymes with AT-rich recognition sites were chosen to maximize cleavage of the AT-rich cpDNA molecule. Resulting cpDNA fragments were resolved in 1.25–4% agarose gels by Southern blot hybridization with ^{32}P-labeled cpDNA sequences. Probes were prepared by extension of random primers (Amersham) annealed to gel-isolated inserts from clones of a *Sac* I *Lactuca* cpDNA library, generously provided by R. K. Jansen and J. D. Palmer, and a 9.0 kb *Pst* I *Petunia* fragment (J. D. Palmer and E. Clark), obtained through K. J. Sytsma. Together these probes represented >99% of the lettuce cpDNA molecule (Fig. 1). Partial restriction mapping of cpDNA from four Hawaiian species with three enzymes revealed no deviations in cpDNA size or structure relative to lettuce (Baldwin et al., unpublished). Restriction site changes were interpreted with reference to standard markers of triple-digested (*Bam* HI, *Eco* RI, *Hin*d III) and single-digested (*Hin*d III) lambda phage DNA. Length mutations were distinguished from restriction site mutations near preexisting sites by comparisons of all digests at the region in question.

Both Wagner and Dollo parsimony were employed in cladistic phylogenetic analyses of the restriction enzyme data set. Wagner parsimony treats all mutations equivalently, whereas Dollo parsimony prohibits certain evolutionary parallelisms—parallel restriction site gains and loss/gains—the least probable homoplasious changes (DeBry & Slade, 1985). A presence/absence matrix of all restriction sites shared by a subset of two or more taxa, including the outgroup species, was analyzed by Wagner parsimony using the PAUP package, version 2.4 (D. L. Swofford, Illinois Natural History Survey). The GLOBAL and MULPARS branch-swapping options and the branch-and-bound (BANDB) option of PAUP were used to search for the shortest topologies. A strict consensus tree of the maximally parsimonious reso-

TABLE 1. Collections of species analyzed for chloroplast DNA restriction site polymorphisms. DNA samples from a given location comprised one individual unless indicated by "(P)" = pooled sample of two to ten individuals from one population. Lowercase letter designations following voucher numbers indicate separate clusters of individuals within a population. Collection numbers in parentheses indicate accessions screened only for select mutations found in the main analysis (see "Materials and Methods"). Collector abbreviations are: *BGB* = *B. G. Baldwin et al.*; *GDC* = *G. D. Carr*. Vouchers are at DAV (*BGB* and *A. Medeiros* collections) or HAW (other collections) unless specifically indicated. [1] (BISH, HAW, US). [2] (PTBG). [3] (BISH, F, US). [4] (BISH, F, HAW, US). [5] (BISH).

Argyroxiphium caliginis C. Forbes—*BGB 660*, Pu'u Kukui, West Maui.

Argyroxiphium grayanum (Hillebrand) Degener—*BGB 661*, Pu'u Kukui, West Maui; *A. Medeiros s.n.*, Haleakala, East Maui.

Argyroxiphium sandwicense DC. subsp. *macrocephalum* (A. Gray) Meyrat—(*GDC 1239*), Haleakala, East Maui.

Argyroxiphium sandwicense DC. subsp. *sandwicense*—*BGB 657* (P), upper Wailuku drainage, Mauna Kea, Hawaii.

Dubautia arborea (A. Gray) Keck—*BGB 527* (P), Pu'u La'au, Mauna Kea, Hawaii; (*BGB 656*), upper Wailuku drainage, Mauna Kea, Hawaii.

Dubautia ciliolata (DC.) Keck subsp. *ciliolata*—*BGB 529* (P), Crater Rim Trail, Kilauea, Hawaii.

Dubautia ciliolata (DC.) Keck subsp. *glutinosa* G. Carr—(*BGB 525*) (P), near Pu'u Huluhulu, Hawaii; (*BGB 659*), upper Wailuku drainage, Mauna Kea, Hawaii.

Dubautia herbstobatae G. Carr—*GDC 1244*, Ohikilolo Ridge, Waianae Range, Oahu.

Dubautia imbricata H. St. John & G. Carr subsp. *imbricata*—*BGB 667*, Wahiawa Mountains, Kauai.

Dubautia knudsenii Hillebrand subsp. *filiformis* G. Carr—(*GDC 1234*), Makaleha Mountain, Kauai.

Dubautia knudsenii Hillebrand subsp. *knudsenii*—*GDC 1047*, Awaawapui Trail near Kokee Road, Waimea, Kauai; (*GDC 946*[1]) (P), Kahuamaa Flat, Kokee Park, Kauai.

Dubautia knudsenii Hillebrand subsp. *nagatae* (H. St. John) G. Carr—(*GDC 1322*a, b) (P), Pihea Trail, Alakai Swamp, Kauai.

Dubautia laevigata A. Gray—*BGB 671*, Kaluapuhi Trailhead, Kokee Park, Kauai.

Dubautia latifolia (A. Gray) Keck—*BGB 675*, (*Flynn 1209*[2]), Makaha Ridge, Kokee Park, Kauai.

Dubautia laxa Hook. & Arn. subsp. *hirsuta* (Hillebrand) G. Carr—(*GDC 833*[3]a, b) (P), Mt. Kaala, Waianae Range, Oahu; (*Flynn 3201*[2]) (P), Pihea Trail, Alakai Swamp, Kauai.

Dubautia laxa Hook. & Arn. subsp. *laxa*—*BGB 662*, Pu'u Kukui, West Maui.

TABLE 1. Continued.

Dubautia linearis (Gaudich.) Keck subsp. *hillebrandii* (H. Mann) G. Carr—*BGB 531* (P), Pohakuloa Military Reservation, Hawaii.

Dubautia linearis (Gaudich.) Keck subsp. *linearis*—(*BGB 516*) (P), Piilani Highway E of Ulupalakua, East Maui.

Dubautia menziesii (A. Gray) Keck—*BGB 522* (P), (*BGB 521*) (P), west slope of Haleakala, East Maui.

Dubautia microcephala Skottsb.—*GDC 1044*[4]a (b) (P), Kahuamaa Flat, Kokee Park, Kauai; (*BGB 671*), Kaluapuhi Trailhead, Kokee Park, Kauai.

Dubautia paleata A. Gray—*GDC 1375*c (a, b, d) (P), Pihea Trail, Alakai Swamp, Kauai.

Dubautia pauciflorula H. St. John & G. Carr—*BGB 668*, Wahiawa Mountains, Kauai.

Dubautia plantaginea Gaudich. subsp. *humilus*—(*GDC 1183*), Black Gorge, West Maui.

Dubautia plantaginea Gaudich. subsp. *plantaginea*—*GDC 1180*, Castle Trail, Koolau Range, Oahu; (*GDC 838*[5]a, b, c) (P), Mt. Kaala, Waianae Range, Oahu.

Dubautia platyphylla (A. Gray) Keck—*BGB 524*, (*BGB 523*) (P), west slope of Haleakala, East Maui.

Dubautia raillardioides Hillebrand—*BGB 670* (P), border of Wahiawa Bog, Kauai; (*GDC 1373*a, b, c) (P), Pihea Trail, Alakai Swamp, Kauai.

Dubautia reticulata (Sherff) Keck—*BGB 664*, Koolau Gap, Haleakala Crater, East Maui.

Dubautia scabra (DC.) Keck subsp. *scabra*—*BGB 530* (P), Crater Rim Trail, Kilauea, Hawaii; (*BGB 526*) (P), near Pu'u Huluhulu, Hawaii.

Dubautia sherffiana Fosb.—*BGB 515* (P), Kamaileunu Ridge, Waianae Range, Oahu; (*GDC 837*a, b, c, d) (P), Mt. Kaala, Waianae Range, Oahu.

Madia bolanderi (A. Gray) A. Gray—*BGB 509*, Lake Tahoe, Sierra Nevada, California.

Raillardiopsis muirii (A. Gray) Rydb.—*BGB 618* (P), Santa Lucia Range, California.

Wilkesia gymnoxiphium A. Gray—*Char 76.022*c (a, b) (P), Iliau Loop Trail, Waimea Canyon rim, Kauai; (*GDC 1157*a, b, c) (P), Waimea Canyon Rd., Kauai.

Wilkesia hobdyi H. St. John—*GDC 1150*, Polihale Ridge, Kauai.

lutions was created using the PAUP CONTREE program. Confidence intervals were derived for each resolved node of the consensus tree using the same data matrix and the PHYLIP statistical package, version 3.1 (J. Felsenstein, Univ. of Washington) bootstrap (BOOT) option (100 replicate runs). Dollo parsimony analysis was conducted with the PHYLIP DOLLOP program from a similar data matrix that indicated double and single fragment profiles for each mutation.

We conducted two genetic distance analyses of the restriction enzyme data. Chloroplast DNA divergence values (p values) for all pairwise species combinations were calculated from restriction site variation following Nei & Li (1979). These estimates of nucleotide substitutions per nucleotide position were used to produce (1) a Fitch & Margoliash (1967) dendrogram (FITCH program of PHYLIP) and (2) a phenetic tree, from a modification of the Fitch-Margoliash algorithm (KITSCH program of PHYLIP) that assumes clocklike evolution of nucleotide sequences.

A preliminary survey of intraspecific cpDNA variation was conducted based on results from the above analyses of one to two populations per species. Additional populations (Table 1) were screened for one or more restriction site mutation(s) unique to a given species in the primary analysis or, where no such mutations existed, for restriction sites most diagnostic for that taxon.

RESULTS

Approximately 875 restriction sites were examined in each cpDNA included in the main analysis. This accounted for 3.7% of the nucleotide sequence in each cpDNA. One of the two copies of the inverted-repeat cpDNA sequence was excluded from these estimates. Fifty-five restriction site differences were detected among the Hawaiian species, 36 (65.5%) of these being shared by a subset of two or more taxa (Table 2). Six additional mutations were common to all species of the silversword alliance and separated them from the two North American outgroup taxa.

Chloroplast DNA divergence within the silversword alliance ranged from 0 to 0.46% of nucleotides (p values; Table 3). For the samples used in the main restriction site analyses, complete interspecific identity in cpDNA was apparent in four instances: *Argyroxiphium caliginis/A. grayanum* (West Maui); *Dubautia imbricata/D. pauciflorula/D. raillardioides*; *D. ciliolata/D. scabra*; and *D. menziesii/D. platyphylla/D. reticulata.* Maximum cpDNA divergence was found within *Dubautia*, exceeding any intergeneric value among the Hawaiian species (Table 4). Genetic distances between the Kauaian rainforest shrub *Dubautia laevigata* and the Mauian bog rosette plants *Argyroxiphium caliginis* and *A. grayanum* ($p = 0.15\%$) were the lowest of any intergeneric cpDNA comparisons, closely approaching the maximum divergence found within the genus *Argyroxiphium* ($p = 0.13\%$), which is conservative in habit.

Chloroplast DNA restriction sites were most highly concentrated in the vicinity of the ribulose bisphosphate carboxylase, large-subunit gene (rbcL; S12) and near the inverted repeat-proximal end of the Compositae inversion (S6; Jansen & Palmer, 1987). Variation in both regions approached one mutation per kilobase (Table 5). Lower occurrences of mutations were found throughout the remainder of the large single-copy (LSC) and small single-copy (SSC; S5) regions (Fig. 1). Only two mutations clearly involved restriction sites within the inverted repeat (Fig. 1), in accord with its marked sequence conservation in other higher plant groups (Palmer & Zamir, 1982; Palmer et al., 1983a, b; Clegg et al., 1984; Sytsma & Gottlieb, 1986). Each of these two site differences yielded alternative fragment profiles with one common band and two others differing to the same extent in opposing digests (Table 2).

Intraspecific cpDNA variation was detected in eight of 15 species screened for specific site mutations found in the main analysis. The following samples lacked mutations unique to their species

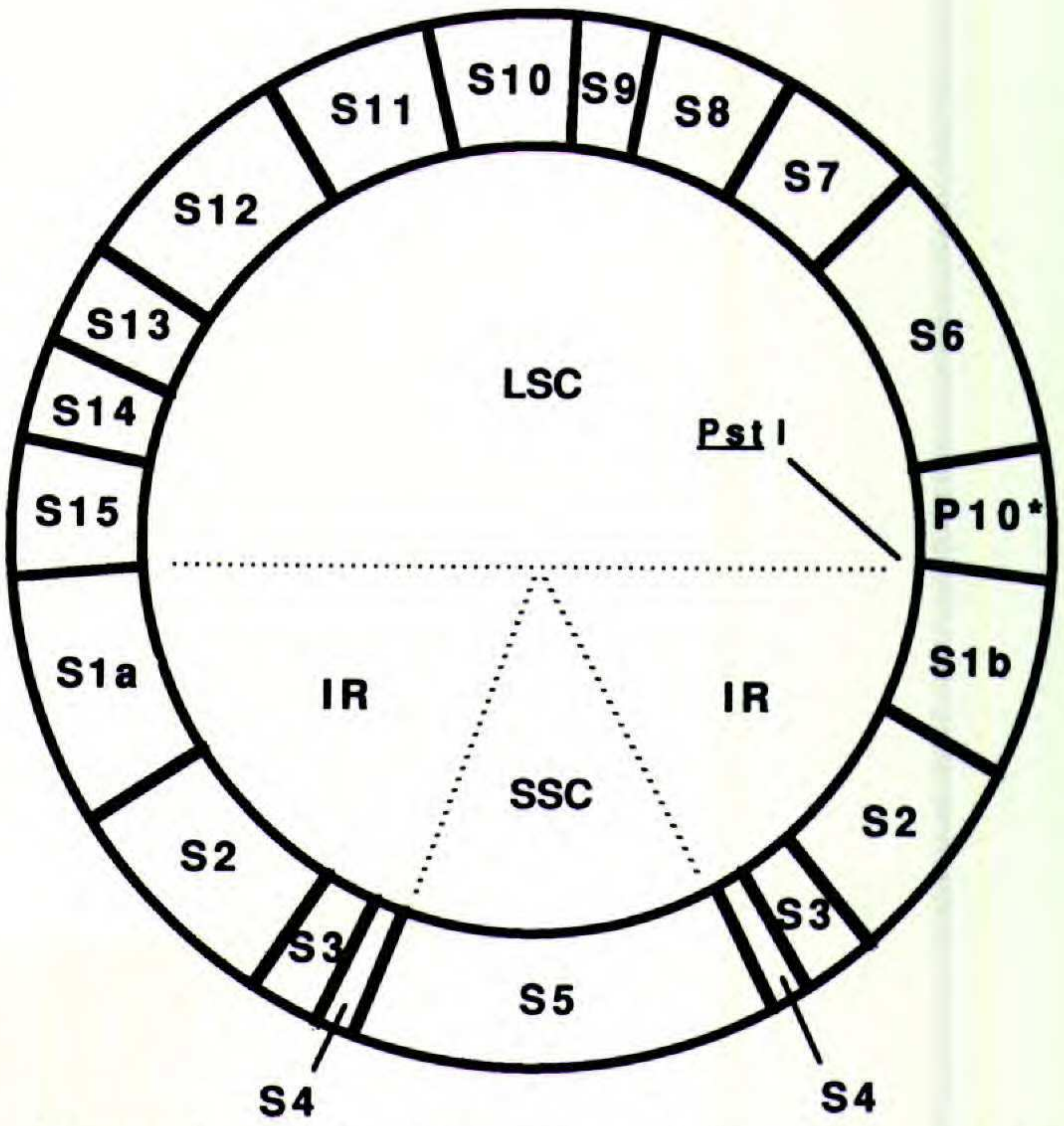

FIGURE 1. Molecular positions of cloned lettuce chloroplast DNA fragments (Jansen & Palmer, 1987) used as Southern blot hybridization probes. Unless otherwise indicated, all lines separating fragments are *Sac* I restriction sites. Fragments less than 1 kilobase in size are not indicated. Designations are: S = *Sac* I; P = *Pst* I; IR = inverted repeat; LSC = large single-copy region; SSC = small single-copy region. Sizes in kilobases of these fragments are: S1a = 12.3; S1b = 8.3; S2 = 9.9; S3 = 3.5; S4 = 1.8; S5 = 18.8; P10* = 7.1; S6 = 14.7; S7 = 7.0; S8 = 6.7; S9 = 3.8; S10 = 6.9; S11 = 7.7; S12 = 10.6; S13 = 4.6; S14 = 5.4; S15 = 6.3. Additionally, gel-isolated probes from digests of S6 (× *Kpn* I) and S12 (× *Xho* I) were used. "P10*" represents only that portion of the 9.0-kb *Pst* I *Petunia* fragment that bridges the inverted repeat and S6.

TABLE 2. Restriction site mutations from ca. 875 restriction sites per cpDNA sampled in the primary analysis of Hawaiian Madiinae and two outgroup taxa (see Table 1). Mutations 1–43 were shared by a subset of two or more species (synapomorphies, including outgroup). The remaining site differences were unique to a single Hawaiian accession in the primary survey (autapomorphies). Mutations unique to the outgroup taxa are in Baldwin (1989). In three instances of outgroup conflict (mutations 9, 14, and 43) the derived condition has been designated based on the resolutions of Wagner and Dollo parsimony with the outgroups included. [1] No mutations were detected with *Hae* II, *Nsi* I, or *Sca* I. [2] The derived fragment condition (in kilobases) appears to the right of the hyphen, following the ancestral state. [3] See Figure 1 for molecular position. Uncertain location of a mutation is indicated by slashes (/) between regions. Hyphens (-) between regions indicate the common domain of both sequences. [4] 1 = *Raillardiopsis muirii*; 2 = *Madia bolanderi*; 3 = *Wilkesia gymnoxiphium*; 4 = *W. hobdyi*; 5 = *Dubautia knudsenii*; 6 = *D. imbricata*; 7 = *D. pauciflorula*; 8 = *D. raillardioides*; 9 = *D. paleata*; 10 = *Argyroxiphium sandwicense*; 11 = *A. grayanum* (E. Maui); 12 = *A. caliginis*; 13 = *A. grayanum* (W. Maui); 14 = *Dubautia plantaginea*; 15 = *D. laxa*; 16 = *D. scabra*; 17 = *D. ciliolata*; 18 = *D. linearis*; 19 = *D. arborea*; 20 = *D. menziesii*; 21 = *D. platyphylla*; 22 = *D. reticulata*; 23 = *D. herbstobatae*; 24 = *D. sherffiana*; 25 = *D. microcephala*; 26 = *D. laevigata*; 27 = *D. latifolia*. [5] Additional fragment profile from a single mutation occurring within the inverted repeat near the LSC borders.

Enzyme[1]	Site mutation[2]	Region[3]	Mutated cpDNA[4]
1. *Bgl*II	1.7 + 0.7-2.4	S13	3–27
2. *Bst*NI	2.8-1.7 + 1.1	S1	25–27
3. *Bst*NI	2.5-2.1 + 0.4	S6	3–27
4. *Bst*NI	0.7 + 0.7-1.4	S11	3, 4
5. *Bst*NI	0.8 + 0.1-0.9	S12	25, 26
6. *Dra*I	1.1 + 0.7-1.8	P10-S6	14–24
7. *Dra*I	1.2 + 1.8-3.0	P10-S6	14–17
8. *Dra*I	1.2-0.7 + 0.5	P10-S6	3–9
9. *Dra*I	1.2 + 1.5-2.7	S6	2, 10
10. *Dra*I	1.5-1.1 + 0.4	S6	14, 27
11. *Dra*I	0.9 + 0.2-1.1	S7	5–9, 14
12. *Dra*I	2.5-2.4 + 0.1	S8	14–24
13. *Dra*I	0.3 + 2.1-2.4	S15	10, 11, 14, 15
14. *Eco*RI	2.6-1.4 + 1.2	S6	1, 14–24
15. *Eco*RI	1.4 + 0.7-2.1	S6	14–24
16. *Eco*RI	2.6 + 2.4-5.0	S6	25, 26
17. *Eco*RI	4.4 + 2.2-6.6	S7	16–19
18. *Eco*RI	3.7-2.5 + 1.2	S12	3, 4
19. *Eco*RI	1.6-1.0 + 0.6	S13	12–15
20. *Eco*RI	1.4 + 1.1-2.5	S5	25, 26
21. *Eco*RV	1.0 + 7.5-8.5	S7	16–19
22. *Eco*RV	7.5-4.2 + 3.3	S8	12, 13
23. *Eco*RV	4.9-4.5 + 0.4	S12/S13	3–27
24. *Hinc*II	2.4 + 4.4-6.8	S11	5–9
25. *Hind*III	1.6 + 1.0-2.6	S7/8	3–27
26. *Hind*III	4.5 + 6.6-11.1	S12	14–24
27. *Hpa*II	3.6 + 0.3-3.9	S5	5–9
28. *Hpa*II	1.0 + 1.0-2.0	S6	3–27
29. *Hpa*II	0.4 + 0.5-0.9	S10	14, 15
30. *Hph*I	1.7 + 1.5-3.2	S6	14–24
31. *Hph*I	3.9 + 0.9-4.8	S8	23, 24
32. *Hph*I	1.1 + 0.7-1.8	S12	16, 17
33. *Ssp*I	1.9 + 1.0-2.9	S1	5–9, 27
34. *Ssp*I	4.0 + 0.1-4.1	S1	16–22
35. *Ssp*I	3.8-3.6 + 0.2	S3/4/5	3–9
36. *Ssp*I	0.4 + 0.4-0.8	P10-S6	3–27
37. *Ssp*I	1.2-1.0 + 0.2	S6	5–8
38. *Xba*I	32.5 + 6.7-39.2	S5	25–27
39. *Xba*I	2.8 + 2.4-5.2	P10	5–9, 19
40. *Xba*I	2.3-1.3 + 1.0	S6	20–22
41. *Xba*I	2.3 + 1.5-3.8	S12	5–9

TABLE 2. Continued.

Enzyme[1]	Site mutation[2]	Region[3]	Mutated cpDNA[4]
42. *Xmn*I	0.5 + 1.3–1.8	S1	3–9
43. *Xmn*I	6.3 + 0.7–7.0 (4.9 + 0.7–5.6)[5]	S1	2, 3, 10, 20–22, 25
44. *Bgl*II	5.7–4.6 + 1.1	S5	25
45. *Bst*NI	1.8 + 0.8–2.6	S6	3
46. *Bst*NI	1.1 + 0.2–1.3	S9	19
47. *Bst*NI	1.1–1.0 + 0.1	S9	9
48. *Bst*NI	0.2 + 0.1–0.3	S12	4
49. *Dra*I	1.2 + 1.1–2.3	P10-S6	27
50. *Dra*I	3.1 + 0.5–3.6	S8	24
51. *Dra*I	8.2 + 0.7–8.9	S10	24
52. *Eco*RI	3.7–2.1 + 1.6	S12	5
53. *Eco*RI	0.9 + 0.2–1.1	S15	11
54. *Hpa*II	2.6–1.5 + 1.1	S12	4
55. *Hph*I	3.9–2.6 + 1.3	S5	4
56. *Nde*I	7.9–4.8 + 3.1	S6	10
57. *Nde*I	1.0 + 1.0–2.0	S14	24
58. *Ssp*I	0.9 + 1.3–2.2	S12	15
59. *Ssp*I	1.0–0.5 + 0.5	S12	27
60. *Xba*I	4.3 + 1.3–5.6	S12/S13	9
61. *Xmn*I	8.6–7.0 + 1.6 (7.9–6.3 + 1.6)[5]	S1	24

in the primary survey, most of these taxa being different subspecies than those analyzed in depth (number in parentheses = mutation designation in left column of Table 2): *Argyroxiphium sandwicense* subsp. *macrocephalum GDC 1239* (56); *Dubautia arborea BGB 656* (46); *D. laxa* subsp. *hirsuta GDC 833*a, b, *Flynn 3201* (58); *D. knudsenii* subsp. *filiformis GDC 1234* (52); *D. knudsenii* subsp. *nagatae GDC 1322*a, b (52); and *Wilkesia gymnoxiphium GDC 1157*a (45). The last sample of *W. gymnoxiphium*, however, indicates intrapopulational cpDNA variation in this taxon. In addition, populations of three species—again, mostly subspecies not sampled in the main analysis—lacked mutations shared between their species and one or more others in the primary survey: *Dubautia ciliolata* subsp. *glutinosa BGB 525*, *BGB 659* (21, 32); *D. linearis* subsp. *linearis BGB 516* (21); *D. plantaginea* subsp. *humilis GDC 1183* (6, 7, 10); and *D. plantaginea* subsp. *plantaginea GDC 838*a, b, c (10). No intraspecific cpDNA variation was observed in *Dubautia latifolia*, *D. menziesii*, *D. microcephala*, *D. paleata*, *D. platyphylla*, *D. raillardioides*, or *D. sherffiana*.

Four length polymorphisms resulting from insertions or deletions of ca. 50–250 nucleotides were resolved within the silversword alliance (Table 6). Three of these four mutations occurred in the LSC region near the inverted repeat (S1 and S6; Fig. 1), regions noted for high incidence of length variation in many plants (Palmer, 1985). The remaining mutation occurred in the SSC region (S5; Fig. 1). Smaller-length polymorphisms could not be well resolved by the gel-systems used. Involvement of the deletion shared by *Dubautia plantaginea* and *D. laxa* in an unscored *Ssp* I site loss in these species was inferred from diminished size of the resulting fragment. This length mutation was absent in the sole accession of *D. plantaginea* subsp. *humilis* (Table 1).

Wagner parsimony analysis of the entire data set (including outgroups) using the PAUP package branch-and-bound (BANDB) option found 1,035 maximally parsimonious trees at 53 steps. The high number of maximally parsimonious trees was attributable to arbitrary resolutions of zero-length branches by PAUP. A PAUP Wagner analysis of these data using the GLOBAL and MULPARS options also found minimum-length trees of 53 steps. Strict consensus trees from the upper limit of 100 trees retained in both PAUP analyses were topologically congruent. The common tree from both approaches is presented in Figure 2. A PAUP Wagner branch-and-bound analysis of the two fully resolved, Kauaian sublineages of this tree (i.e., *Wilkesia/Dubautia* sister group; *Dubautia laevigata/D. latifolia/D. microcephala*) indicated that each represented the most parsimonious topology. Wagner branch-and-bound analysis of the

TABLE 3. Pairwise cpDNA divergence among primary Hawaiian Madiinae samples and outgroup taxa (see Table 1). Values in the upper right half of matrix are numbers of mutations separating two cpDNAs from ca. 875 restriction sites sampled in each. Numbers of nucleotide substitutions per nucleotide position, or p values (Nei & Li, 1979), appear in the lower left half of matrix as $100p$. Samples corresponding to the number designations along the left and top matrix borders are: 1 = *Raillardiopsis muirii*; 2 = *Madia bolanderi*; 3 = *Wilkesia gymnoxiphium*; 4 = *W. hobdyi*; 5 = *Dubautia knudsenii*; 6 = *D. imbricata/D. pauciflorula/D. raillardioides*; 7 = *D. paleata*; 8 =

	1	2	3	4	5	6	7	8	9	10
1	—	19	25	26	29	28	29	22	20	20
2	0.398	—	18	21	24	23	24	13	15	15
3	0.525	0.377	—	5	12	11	12	9	9	9
4	0.546	0.440	0.104	—	13	12	13	12	10	10
5	0.611	0.504	0.250	0.271	—	1	4	15	13	13
6	0.589	0.483	0.229	0.250	0.021	—	3	14	12	12
7	0.611	0.504	0.250	0.271	0.083	0.062	—	15	13	13
8	0.461	0.271	0.187	0.250	0.313	0.292	0.313	—	4	6
9	0.419	0.313	0.187	0.208	0.271	0.250	0.271	0.083	—	4
10	0.419	0.313	0.187	0.208	0.271	0.250	0.271	0.125	0.083	—
11	0.589	0.525	0.398	0.419	0.440	0.419	0.440	0.292	0.250	0.250
12	0.568	0.504	0.377	0.398	0.461	0.440	0.461	0.271	0.229	0.229
13	0.568	0.504	0.377	0.398	0.461	0.440	0.461	0.313	0.271	0.271
14	0.525	0.461	0.334	0.355	0.419	0.398	0.419	0.271	0.229	0.229
15	0.568	0.504	0.377	0.398	0.419	0.398	0.419	0.313	0.271	0.271
16	0.525	0.419	0.292	0.355	0.419	0.398	0.419	0.229	0.229	0.229
17	0.483	0.419	0.292	0.313	0.377	0.355	0.377	0.229	0.187	0.187
18	0.568	0.504	0.377	0.398	0.461	0.440	0.461	0.313	0.271	0.271
19	0.525	0.377	0.250	0.313	0.377	0.355	0.377	0.187	0.187	0.187
20	0.483	0.377	0.250	0.271	0.334	0.313	0.334	0.187	0.146	0.146
21	0.504	0.398	0.271	0.292	0.313	0.292	0.313	0.208	0.166	0.166

D. plantaginea/D. laxa/D. scabra/n = 13 *Dubautia* sublineage yielded nine maximally parsimonious resolutions, all concordant with the consensus topology. Bootstrap (PHYLIP BOOT program) confidence intervals for resolved consensus tree branches ranged from 59% to 100% (Fig. 2).

The consensus phylogeny, excluding the outgroups, had a consistency index (C.I.; Kluge & Farris, 1969) of 0.85 (15% parallelism) with 48 site losses and 24 site gains, including nine parallel losses and two parallel gains. Exclusion of two highly homoplasious characters (13 and 43; Table 2) resulted in the same consensus resolution with a C.I. of 0.91 (9% parallelism). These C.I. values include autapomorphies.

The high incidence of parallelism largely involved mutation "hotspot" centers at both ends of the LSC region, near the inverted repeat (Palmer, 1985). The predominance of parallelism involving the *Dubautia plantaginea* and *D. laxa* clade is not readily explained by length polymorphisms, introgression, or weak placement in the tree. Absence of a parallel site gain (10; Table 2) in additional populations of *D. plantaginea* suggests that this mutation indeed arose twice.

Dollo parsimony (PHYLIP package, DOLLOP program) analyses all yielded results topologically congruent with the strict consensus tree (Fig. 2). These trees, however: (1) consistently placed the *D. plantaginea/D. laxa* branch basal to *D. herbstobatae* and *D. sherffiana*, thereby resolving the *n* = 13 *Dubautia* species and *D. scabra* as a monophyletic group, and (2) placed *A. sandwicense* in a sister-group relationship with East Mauian *A. grayanum*.

Fitch-Margoliash genetic distance analysis yielded results completely harmonious with both the Wagner strict consensus tree (Fig. 2) and the Dollo parsimony results. The dendrogram produced was the best topology of all trees examined, i.e., in being most compatible with measured species divergences (Table 3). The average "standard deviation" of this outcome was 4.53%. Though fully resolved, this result was only slightly better than alternative conformations differing at nodes unresolved in the consensus tree. Accordingly, the entire dendrogram topology is not presented.

Phenetic analysis of the genetic distance values using the PHYLIP package, KITSCH program gave results contrary to the consensus tree (Fig. 2). Unlike all other analyses, the best phenetic resolution (average "standard deviation" = 26.06%) placed the following species, separated

TABLE 3. Extended.
Argyroxiphium sandwicense; 9 = *A. grayanum* (East Maui); 10 = *A. caliginis/A. grayanum* (West Maui); 11 = *Dubautia plantaginea*; 12 = *D. laxa*; 13 = *D. scabra/D. ciliolata*; 14 = *D. linearis*; 15 = *D. arborea*; 16 = *D. menziesii/D. platyphylla/D. reticulata*; 17 = *D. herbstobatae*; 18 = *D. sherffiana*; 19 = *D. microcephala*; 20 = *D. laevigata*; 21 = *D. latifolia*.

11	12	13	14	15	16	17	18	19	20	21
28	27	27	25	27	25	23	27	25	23	24
25	24	24	22	24	20	20	24	18	18	19
19	18	18	16	18	14	14	18	12	12	13
20	19	19	17	19	17	15	19	15	13	14
21	22	22	20	20	20	18	22	18	16	15
20	21	21	19	19	19	17	21	17	15	14
21	22	22	20	20	20	18	22	18	16	15
14	13	15	13	15	11	11	15	9	9	10
12	11	13	11	13	11	9	13	9	7	8
12	11	13	11	13	11	9	13	9	7	8
—	3	9	9	11	9	7	11	19	17	16
0.062	—	8	8	10	8	6	10	18	16	17
0.187	0.166	—	2	4	6	6	10	18	16	17
0.187	0.166	0.041	—	2	4	4	8	16	14	15
0.229	0.208	0.083	0.041	—	6	6	10	18	16	17
0.187	0.166	0.125	0.083	0.125	—	4	8	14	14	15
0.146	0.125	0.125	0.083	0.125	0.083	—	4	14	12	13
0.229	0.208	0.208	0.166	0.208	0.166	0.083	—	18	16	17
0.398	0.377	0.377	0.334	0.377	0.292	0.292	0.377	—	2	9
0.355	0.334	0.334	0.292	0.334	0.292	0.250	0.334	0.041	—	7
0.334	0.355	0.355	0.313	0.355	0.313	0.271	0.355	0.187	0.146	—

by slash marks, in sister-group relationships: (1) the species of *Argyroxiphium/Dubautia laevigata*; (2) *Dubautia menziesii, D. platyphylla, D. reticulata/D. sherffiana*; and (3) *Dubautia herbstobatae/D. arborea, D. ciliolata, D. linearis, D. scabra*.

DISCUSSION

CHLOROPLAST DNA DIVERGENCE

Chloroplast DNA nucleotide sequence divergence within the silversword alliance ($p = 0$–0.46%) was similar to that of the least divergent continental genera that have been documented; e.g., *Helianthus* ($p = 0.3\%$–0.4%; Rieseberg et al., 1988), *Lycopersicon* ($p = 0$–0.7%; Palmer & Zamir,

TABLE 4. Limits of intrageneric and intergeneric chloroplast DNA divergence detected in Hawaiian Madiinae. Divergence values (p = nucleotide substitutions per nucleotide position) are from Table 3.

	Minimum (p)	Maximum (p)
Argyroxiphium	0	0.13%
Dubautia	0	0.46%
Wilkesia	0.10%	0.10%
Argyroxiphium/Dubautia	0.15%	0.31%
Argyroxiphium/Wilkesia	0.19%	0.25%
Dubautia/Wilkesia	0.23%	0.42%
Total	0	0.46%

TABLE 5. Molecular distribution of chloroplast DNA restriction site mutations in Hawaiian Madiinae. Regions refer to probe sequences flanked by *Sac* I or *Pst* I restriction sites (see Fig. 1). [1] Region of the 9.0-kb *Pst* I sequence between the inverted repeat and S6. [2] Underestimated owing to unscored *Dra* I mutations not well resolved with S5 probe.

Regions	Numbers of mutations	Mutations/ kilobase
S6 (14.7 kb)	14	0.95
S12 (10.6 kb)	10–11	0.94–1.04
S8 (6.7 kb)	4	0.60
S9 (3.8 kb)	2	0.53
S1 (12.3 kb)	6	0.49
S7 (7.0 kb)	3	0.43
S15 (6.3 kb)	2	0.32
S10 (6.9 kb)	2	0.29
S5 (18.8 kb)	5–6	0.27–0.32[2]
S11 (7.7 kb)	2	0.26
S13 (4.6 kb)	1–2	0.22–0.44
P10[1] (7.1 kb)	1	0.14

TABLE 6. Chloroplast DNA length mutations within the silversword alliance. Additional insertions and deletions smaller than 100 nucleotide pairs were not clearly resolved using the gel system employed. [1] Samples from the primary restriction site analysis (Table 1). [2] See Figure 1. [3] Direction of mutation based on outgroup comparison. [4] Same approximate map position but visibly larger insertion in *D. herbstobatae* relative to *D. sherffiana*.

Taxon[1]	Region[2]	Type of mutation[3]	Size of mutation
D. plantaginea/ *D. laxa*	S1	deletion	100–250 bp
D. paleata	S5	insertion	50–150 bp
D. herbstobatae[4]	P10/S6	insertion	50–100 bp
D. sherffiana[4]	P10/S6	insertion	50–100 bp

1982), *Lisianthius* ($p = 0$–0.3%; Sytsma & Schaal, 1985), and *Pennisetum* ($p = 0$–0.6%; Clegg et al., 1984). Overall allozymic variation among Hawaiian Madiinae also approximated that of some continental genera (Witter & Carr, 1988). Both measures of genetic differentiation, however, indicate higher levels of molecular divergence in the silversword alliance than in previously investigated autochthonous oceanic plant groups. For example, preliminary surveys of cpDNA variation in Hawaiian *Tetramolopium* (Asteraceae) and *Dendroseris* (Asteraceae) from the Juan Fernandez Islands revealed no polymorphism (Crawford et al., 1987), though fewer species and restriction sites were examined than in the present study.

Comparisons of cpDNA divergence values presented here with those measured among North American Madiinae (Baldwin, 1989) revealed expected and surprising patterns. Higher overall levels of divergence were found among the continental perennial taxa relative to within the silversword alliance. This accords with evidence indicating the mainland group is ancestral to the Hawaiian species (Baldwin, 1989). Maximum cpDNA divergence within *Dubautia* ($p = 0.46\%$), however, was similar to that between both North American outgroup species in this study (*Madia bolanderi* and *Raillardiopsis muirii*) and Hawaiian Madiinae. In fact, cpDNA genetic distances between North American *Madia bolanderi* and species of *Argyroxiphium* ($p = 0.27\%$–0.31%) were considerably less than in some comparisons within *Dubautia* (Table 7). These results suggest closer relationship of North American Madiinae to the silversword alliance than indicated by phenotypic considerations.

Patterns of sequence divergence in the silversword alliance (Fig. 2) suggest that cpDNA evolution has been irregular in this group. Conflicting results from the two genetic distance programs FITCH and KITSCH indeed provide evidence against operation of a cpDNA molecular clock among these species. In particular, lack of cpDNA sequence divergence was apparent in *Argyroxiphium*, comprising the silverswords and greenswords. Allozymic divergence within *Argyroxiphium* was likewise low (Witter, 1986). The mechanics of cpDNA evolution are still too little understood (Birky, 1988) to address the lack of cpDNA divergence in *Argyroxiphium*, i.e., does highly irregular or long-delayed sexual reproduction in this genus influence the rate of cpDNA evolution?

COMPREHENSIVE PATTERN OF ADAPTIVE RADIATION

Origin of the silversword alliance from a single colonizing event to the Hawaiian archipelago, or at most a few events closely spaced in time, is strongly suggested by the high confidence (89%) that this group is monophyletic (Fig. 2). This is completely concordant with the interfertility and cytogenetic cohesiveness of these species. Further, the basal, unresolved hexachotomy of the consensus tree is consistent with a rapid radiation of major lineages early in the history of the alliance. This pattern could alternatively be explained by an increase in the rate of cpDNA evolution through time or a period of extensive hybridization and introgression that led to fixation of one cpDNA type throughout Hawaiian Madiinae. The wealth of systematic data for the silversword alliance, however, supports close genetic affinities among all members despite extreme phenotypic and ecological differences, exactly as would be expected in a group that underwent explosive diversification. This explanation has also been proposed for similar unresolved cpDNA polychotomies among sections of *Clarkia* (Sytsma & Smith, 1988) and in Oncidiinae (reviewed in Palmer et al., 1988).

BIOGEOGRAPHIC CONSIDERATIONS

A consistent feature of the cpDNA consensus tree topology is the strong positive correlation between species affinity and geographic proximity. In almost every case where resolution was available, individual species were most closely grouped with others occurring on the same island. This correlation suggests two hypotheses. First, this pattern alone suggests that few successful interisland colonizations led to subsequent species radiations. Second, extensive introgressive hybridization or hybrid

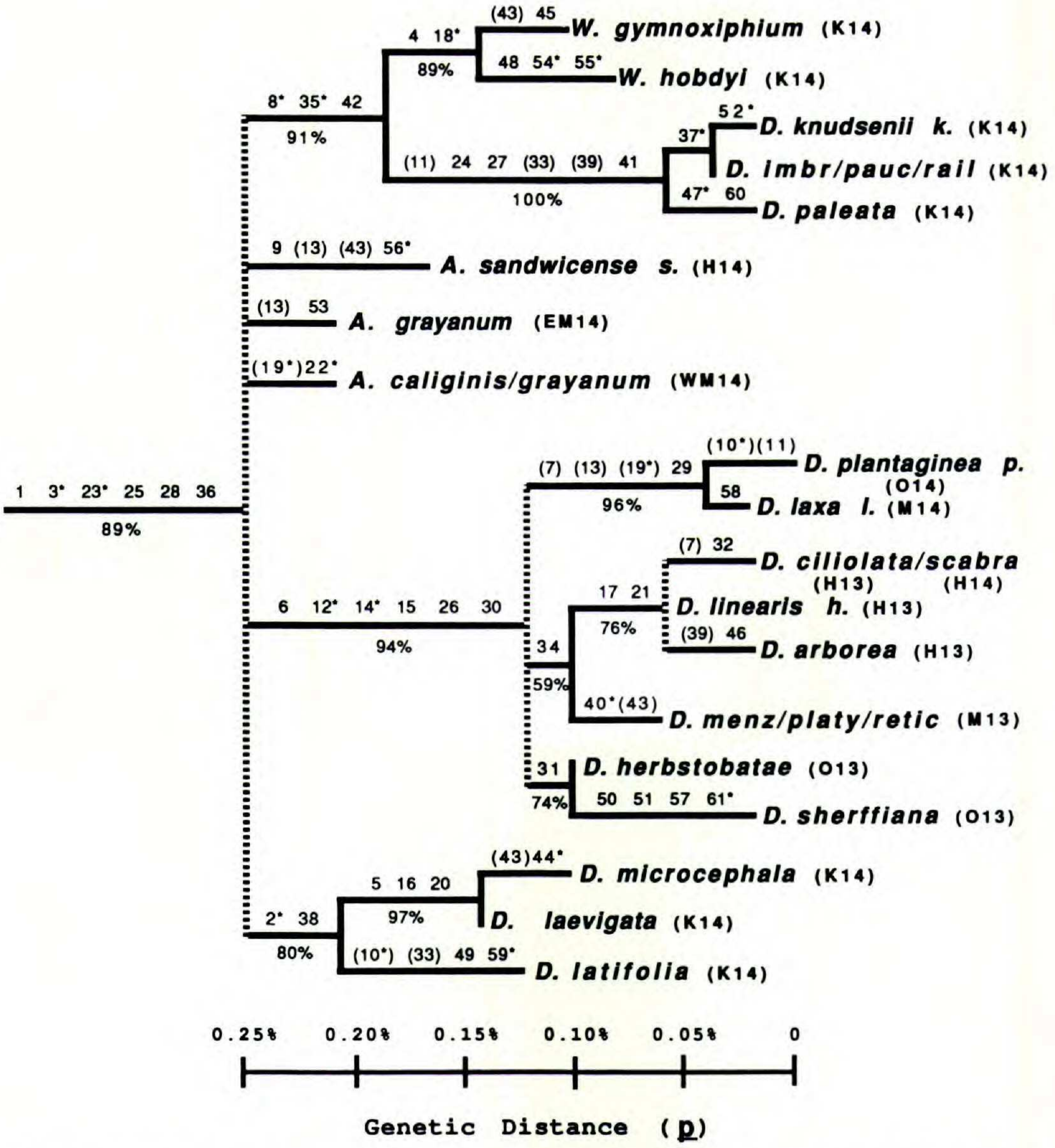

FIGURE 2. Strict consensus tree from Wagner parsimony analyses of a chloroplast DNA restriction site survey of Hawaiian Madiinae (see Table 1). Evolutionary polarity was determined by global parsimony using two North American tarweeds, *Madia bolanderi* and *Raillardiopsis muirii*. The best resolutions from Dollo parsimony and the Fitch-Margoliash genetic distance algorithm were congruent with this topology. Numbers above the horizontal branches refer to mutation designations in the left column of Table 2. Asterisks (*) denote restriction site gains; parentheses ( ) enclose characters showing evolutionary parallelism or homoplasy. This parallelism accounts for slight deviation between genetic distances (p) here and those in Table 3. Bootstrap confidence intervals are given below the horizontal branches as percentages. Broken lines indicate unresolved polychotomies. Taxon abbreviations are: *A.* = *Argyroxiphium*; *D.* = *Dubautia*; *W.* = *Wilkesia*; *D. imb/pauc/rail* = *Dubautia imbricata*, *D. pauciflorula*, and *D. raillardioides*; *D. menz/platy/retic* = *Dubautia menziesii*, *D. platyphylla*, and *D. reticulata*. See Table 1 for additional species data. Abbreviations following taxa names give the island of sample origin followed by the haploid chromosome number: K = Kauai; O = Oahu; M = Maui; EM = East Maui; WM = West Maui; H = Hawaii (Big Island). Only five species (*A. sandwicense*, *D. laxa*, *D. linearis*, *D. plantaginea*, *D. scabra*) have known distributions on multiple islands (Carr, 1985).

speciation on a given island may have resulted in the observed cpDNA relationships. The most widespread species in the alliance, *Dubautia plantaginea* and *D. laxa*, found on most of the Hawaiian Islands, are important for assessing these hypotheses. On different islands these species could be introgressed with or ancestral to local endemics. The consensus tree topology (Fig. 2) does not preclude one of these two taxa from being an old, genetically polymorphic species that founded existing lineages. Preliminary evidence of considerable intraspecific cpDNA variation between *D. plantaginea* on Maui and Oahu is therefore intriguing.

Another biogeographically interesting cpDNA pattern was resolved strictly among the *Dubautia* species having a chromosome number of $n = 13$ (hereafter "$n = 13$ *Dubautia*"). Carr & Kyhos (1986) argued from cytology, morphology, and distribution that these species form a monophyletic assemblage derived from an ancestral $n = 14$ *Dubautia* genome by a single reciprocal translocation followed by dysploidy. Close relationship of all $n = 13$ *Dubautia* taxa is also evident from their

TABLE 7. Lowest chloroplast DNA divergence values between silversword alliance species and the North American tarweed outgroup taxa, *Madia bolanderi* and *Raillardiopsis muirii*. These genetic distances (p values, Table 3) are equivalent to or actually exceeded by some among Hawaiian Madiinae.

Divergence from *Madia bolanderi* (p)		Divergence from *Raillardiopsis muirii* (p)	
Argyroxiphium sandwicense	0.27%	*Argyroxiphium caliginis*	0.42%
Argyroxiphium caliginis	0.31%	*Argyroxiphium grayanum*	0.42%
Argyroxiphium grayanum	0.31%	*Argyroxiphium sandwicense*	0.46%
Wilkesia gymnoxiphium	0.38%		
Dubautia laevigata	0.38%		
Dubautia microcephala	0.38%		
Dubautia latifolia	0.40%		
Dubautia reticulata	0.42%		
Dubautia menziesii	0.42%		
Dubautia platyphylla	0.42%		
Dubautia herbstobatae	0.42%		
Wilkesia hobdyi	0.44%		
Dubautia linearis	0.46%		

average identity in allozymes, comparable to that of a single mainland species (Witter & Carr, 1988). Accordingly, except for the position of *D. scabra* (see below), all Dollo parsimony analyses of the cpDNA data resolved $n = 13$ *Dubautia* as monophyletic. Aside from the basal phylogenetic ambiguity, the cpDNA consensus tree (Fig. 2) is similarly consistent with monophylesis of $n = 13$ *Dubautia*.

A unidirectional, west-to-east pattern of colonization from Oahu to progressively younger islands by members of the $n = 13$ *Dubautia* lineage is suggested by cpDNA phylogenetic data (Fig. 2), albeit with limited statistical confidence from bootstrap analysis. This migratory trend was proposed for the silversword alliance in general by Carr et al. (1989) and is supported by allozymic data at the intraspecific level in some *Dubautia* taxa (Witter, 1986). The same pattern of migration was inferred from polytene chromosome analysis in Hawaiian picture-winged *Drosophila* (Carson, 1983).

Instances of back-migration from younger to older islands cannot, however, be dismissed for $n = 13$ *Dubautia*. Hybridization between such immigrants and established $n = 13$ species could result in fixation of a native cpDNA genome in the alien taxon. Natural interspecific hybridization among $n = 13$ *Dubautia*, resulting in highly fertile hybrids, has been documented for eight species combinations (Carr, 1985). Comparisons of phylogenies based on biparentally and uniparentally inherited characters are critical to understanding the influence of hybridization on these species. Allozymic (Witter, 1986; Witter & Carr, 1988) and cpDNA data both indicated monophylesis of the two Oahu $n = 13$ species, *D. herbstobatae* and *D. sherffiana*, but differed slightly in resolution of Mauian and Hawaiian $n = 13$ *Dubautia* relationships. *Dubautia linearis* subsp. *hillebrandei* was placed with *D. arborea* and *D. ciliolata* in the cpDNA consensus tree (Fig. 2) but was excluded from the allozyme-defined clade comprising *D. arborea*, *D. ciliolata*, *D. menziesii*, and *D. platyphylla*. This conflict could indicate that the cpDNA of *D. linearis* subsp. *hillebrandei* was obtained from another Hawaiian *Dubautia* taxon by hybridization. Such hybridization may explain (1) extreme disparity in flavonoids between *D. linearis* subsp. *hillebrandei* on Hawaii and subsp. *linearis* on Maui (W. J. Crins and B. A. Bohm, pers. comm.) and (2) anatomical similarities of *D. linearis* subsp. *hillebrandei* to *D. arborea* (Carlquist, 1959a). Intraspecific cpDNA polymorphism within $n = 13$ *Dubautia* species, including *D. linearis*, lends promise that additional sampling and cpDNA analyses accompanied by nuclear DNA studies will enhance resolution of biogeographic relationships and hybridization among these species.

PHYLOGENETIC IMPLICATIONS

I. *Wilkesia* and Kauaian *Dubautia*

A well-resolved cpDNA sublineage within the silversword alliance supports monophylesis of *Wilkesia* and of five Kauaian *Dubautia* species (*D. knudsenii*, *D. imbricata*, *D. paleata*, *D. pauciflorula*, and *D. raillardioides*). *Wilkesia* is also delimited by unique alleles and high identities in allozymes (Witter, 1986; Witter & Carr, 1988). Among Kauaian *Dubautia*, distribution of unique allozymes suggests close relationship between *D.*

knudsenii and *D. pauciflorula* and between *D. paleata* and *D. raillardioides* (Witter & Carr, 1988).

Surprisingly, *Wilkesia* and the above *Dubautia* species together comprise a single lineage. Both species in *Wilkesia*, *W. gymnoxiphium* and *W. hobdyi*, have large, yuccalike rosettes born on narrow, naked stems. Leaf venation in *W. gymnoxiphium* closely approximates the parallel-vein condition seen in many monocots (Carlquist, 1959a; Kim, 1987). Unlike these unusual Kauaian xerophytes, members of the cpDNA sister group of *Wilkesia* comprise a *Dubautia* lineage of Kauaian rainforest trees and shrubs having relatively typical dicot habits and leaf venation. The extensive phenotypic contrasts between these two groups were reviewed by Carr (1985). Close cpDNA affinity between *Wilkesia* and these *Dubautia* species represents a striking example of inconsistency between relative rates of cpDNA and phenotypic evolution. The taxonomic implication of this finding is that *Dubautia* is either paraphyletic (i.e., *Wilkesia* is derived from *Dubautia*) or polyphyletic, assuming that ancient hybridization does not account for this cpDNA pattern. The distribution of genome 1 species in *Dubautia* (discussed below), however, argues against polyphylesis of this genus (Carr & Kyhos, 1986).

The other lineage of endemic Kauaian *Dubautia* taxa, comprising *D. latifolia*, *D. laevigata*, and *D. microcephala*, is well delineated by two basal restriction site gains. These rainforest species are to varying degrees sympatric with members of the previous *Dubautia* group. This resolution places *D. latifolia*, the only liana in the silversword alliance, in close relationship with *D. laevigata*, a large shrub, and *D. microcephala*, a small tree.

II. *Argyroxiphium*

Resolved patterns of cpDNA relationship within *Argyroxiphium* (silverswords and greenswords) strongly suggest that either hybridization has been an active force in this genus or extreme evolutionary parallelism in morphology or cpDNA has occurred. These data place a bog greensword (*A. grayanum*) from West Maui in closer relationship to the sympatric bog silversword *A. caliginis* than to a presumably conspecific greensword on East Maui. In addition, a weaker cpDNA sister-species relationship between East Mauian *A. grayanum* and the parapatric silversword *A. sandwicense* was consistently resolved by Dollo, but not Wagner, parsimony.

Intergeneric hybrid origin of the allopatric and somewhat morphologically distinct greenswords on East and West Maui via different stabilized combinations of silversword and *Dubautia* species would account for these cpDNA patterns. This hypothesis is supported by the remarkably greensword-like appearance of the artificial F_1 hybrid *Argyroxiphium sandwicense* × *Dubautia plantaginea* (Carr & Kyhos, unpublished). The latter species occurs sympatrically with greenswords in both regions (Carr, 1985).

Alternatively, these cpDNA patterns may indicate extensive introgression resulting in movement of cpDNA from silversword to greensword or perhaps the reverse. Natural hybrids have been reported between *A. caliginis* and *A. grayanum* at Pu'u Kukui on West Maui (Carr, 1985), where these taxa were sampled. In addition, contact between *A. sandwicense* and *A. grayanum* on East Maui may have occurred until recently, prior to the decimation of these plants by feral mammals.

III. *Dubautia scabra* and $n = 13$ *Dubautia*

A compelling argument for introgressive cpDNA transfer can be made from the cpDNA relationship between *D. scabra* and $n = 13$ *Dubautia*. In contrast with incomplete cytogenetic knowledge of *Wilkesia*, *Argyroxiphium*, and Kauian *Dubautia* taxa, extensive chromosomal data indicate genomic uniformity among $n = 13$ *Dubautia* species (Carr & Kyhos, 1986). These last taxa appear to be derived from the $n = 14$ *D. scabra* by a reciprocal chromosomal translocation and can be linked through *D. scabra* to other $n = 14$ genomes. In addition, *D. scabra* and $n = 13$ *Dubautia* are markedly similar in morphology and distribution, reflected by their exclusive taxonomic segregation as *Dubautia* sect. *Railliardia*. This evidence suggests *D. scabra* is a likely sister taxon to $n = 13$ *Dubautia*. Terminal placement of *D. scabra* in the $n = 13$ *Dubautia* cpDNA lineage and cpDNA identity of *D. scabra* and *D. ciliolata* at Kilauea are therefore unexpected. If these cpDNA and cytogenetic results both reflect organismic phylogeny, one of two improbable scenarios must have occurred: (1) a centromere and two chromosome arms similar to those originally lost by the $n = 13$ group arose, concomitant with a reciprocal translocation, to re-create the $n = 14$ *D. scabra* genome by ascending dysploidy (i.e., *D. scabra* is derived from $n = 13$ *Dubautia*), or (2) multiple independent origins of the $n = 13$ genome occurred from *D. scabra* or a genomically identical progenitor (i.e., *D. scabra* or an immediate predecessor is ancestral to $n = 13$ *Dubautia*).

Field studies indicate that in nature *D. scabra* hybridizes extensively with $n = 13$ *Dubautia* species to produce highly fertile F_1 generations (Carr, 1985). Six species combinations of this type are known. *Dubautia scabra* and *D. ciliolata* occur in sympatry in the two areas where the former was sampled. In addition to apparent F_1 hybrids occupying intermediate habitats at both sites, Crins et al. (1988) reported what appear to be introgressed flavonoid profiles from individuals at the Pu'u Huluhulu site. Uniformity of cpDNA between these species at Kilauea could have resulted from such introgression.

The unexpected cpDNA affinity of *D. scabra* ($n = 14$) to $n = 13$ *Dubautia* taxa may, however, be the result of earlier hybridization on Hawaii. Intraspecific variation between *D. ciliolata* subsp. *glutinosa* at Pu'u Huluhulu and *D. ciliolata* subsp. *ciliolata* at Kilauea is not mirrored by *D. scabra*, which is uniform at examined restriction sites in all of several plants from both localities. Chloroplast DNA transfer from *D. scabra* to *D. ciliolata* therefore appears a more likely explanation for this pattern than the reverse. In short, introgression may account for cpDNA identity between *D. scabra* and *D. ciliolata* at Kilauea, but would not account for $n = 13$ *Dubautia* cpDNA in *D. scabra*. The evolutionary history of these species is being further analyzed, in part, by examination of cpDNA from *D. scabra* on the older island Maui, where the Hawaiian $n = 13$ taxa *D. ciliolata*, *D. arborea*, and *D. linearis* subsp. *hillebrandei* do not occur.

IV. *Dubautia* genome 1

The distribution of *D. knudsenii*, *D. plantaginea*, *D. laxa*, and *D. microcephala* on the cpDNA tree conflicts with their common possession of genome 1 (Carr & Kyhos, 1986). Ancient and recent hybridization together could account for these discrepancies, i.e., cpDNA may not reflect phylogeny. It may be significant, however, that (1) cytogenetic variation within genome 1 species is imperfectly known, with sampling limited to single collections from one or two populations, and (2) these taxa are all found on the oldest Hawaiian island, Kauai, concordant with genomic antiquity.

The existence within individual species of two or more genome types differing by chromosomal rearrangements, a situation well documented in North American Madiinae (Clausen, 1951; Carr, 1975; Carr & Carr, 1983), could explain the cpDNA results. For example, *Dubautia plantaginea* and *D. laxa* are a minimum of two chromosomal interchanges removed from the group with which they showed cpDNA alliance and with which *D. plantaginea* had high genetic identity in allozymes (Witter & Carr, 1988): *D. scabra*/$n = 13$ *Dubautia*. A structurally intermediate genome in one of the first two species could explain evolutionary transition from genome 1 to the *D. scabra* genome. Although no intraspecific chromosomal variation is presently known from Hawaiian Madiinae, such undetected heterogeneity may account for unexpected cytogenetic results in *Argyroxiphium* (Carr & Kyhos, 1986). Expanded cpDNA investigations and molecular analyses of nuclear DNA are needed to clarify the relative extent to which hybridization, intraspecific differentiation, and evolutionary parallelism have influenced phylogenetic patterns in this remarkable group of plants.

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